Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Applicants have amended claims 1, 48, and 71, and canceled claim 72. The above amendments to claims 1, 48, and 71 are supported in the specification at page 6, line 23 to page 7, line 6, Figures 1A-B, and page 9, lines 3-7. Claims 1, 6, 11, 16, 48, 49, 51-54, 66-71, and 73-82 are pending.

The rejection of claims 1, 11, 66-68, 70, 73-76, and 81-82 under 35 U.S.C. § 102(b) for anticipation by Gong et al., "Structure of a Biological Oxygen Sensor: A New Mechanism for Heme-Driven Signal Transduction," *Proc Natl Acad Sci USA* 95:15177-15182 (1998) ("Gong") is respectfully traversed in view of the above amendments.

Applicants have determined that the publication date of Gong was December 22, 1998, which is less than a year prior to the filing of the instant application. Therefore, Gong can only be used as a prior art reference under 35 U.S.C. § 102(a) against the present invention. Nevertheless, applicants submit that Gong cannot anticipate the present invention because Gong fails to teach or suggest each and every limitation of the claimed invention.

Gong teaches the recombinant expression and purification of the FixL protein from *B. japonicum*. The FixL proteins are biological oxygen sensors that restrict the expression of specific genes to hypoxic conditions (Gong at abstract). The FixL protein of Gong contains a heme-binding domain at its N-terminus and a histidine kinase domain at or near the C-terminus (Gong at 15178, rt. col., 2nd full para., and Figure 1). In the presence of bound ligand (i.e., O_2), the heme domain of FixL regulates the histidine kinase domain, which transduces its signal by a conserved phosphoryl transfer mechanism (Gong at 15177, rt. col., 1st sentence). As described by Freitas et al., "The Diversity of Globin-Coupled Sensors," *FEBS Letters* 552:99-104 (2003) ("Freitas"), attached hereto as Exhibit 1, there are six known types of globin-coupled sensors, which can be grouped into the aerotactic and the gene regulating subfamilies based on whether the changes in intracellular gas concentrations sensed by the heme moiety result in either aerotaxis or gene regulation (Freitas at 99, left col., 1st para.). Members of the aerotactic subfamily, which is made up solely of the HemAT proteins, possess an N-terminal globin domain and a C-terminal signaling domain that mediates aerotaxis through a C-terminal methyl-accepting chemotaxis protein ("MCP")-like domain (Freitas at 101, rt. col., 3rd full para.; *see also* present application at pg. 6, line 3-13).

In contrast, the C-terminal signaling domain of FixL proteins belong to the gene regulation subfamily (Freitas at 99, left col., 1st para.). Specifically, histidine kinase of FixL binds heme at an N-terminal PAS domain and controls transcription of oxygen-sensitive genes by its response regulator, FixJ (Freitas at 99, rt. col., last two sentences of para. bridging left and rt. cols.). Phosphorylated FixJ acts as the transcriptional activator and permits transcription of the *fix* genes (*id*.), which leads to nitrogen fixation gene expression in the organism (Gong et al., "New Mechanistic Insights from Structural Studies of Oxygen-Sensing Domain of *Bradyrhizobium japonicum* FixL," *Biochemistry* 39:3955-3962 (2000) at 3955, left col., 1st para., attached hereto as Exhibit 2).

In contrast, claim 1 of the present invention is drawn to an "isolated complex comprising a heme binding protein complexed with a porphyrin, wherein said complex reversibly binds oxygen with a low affinity and wherein said protein comprises (i) a heme binding domain ... and (ii) an aerotaxis signaling domain that has at least 30% identity to SEQ ID NO: 79." Because the FixL protein of Gong does not contain an aerotaxis signaling domain, let alone an aerotaxis signaling domain that has at least 30% identity to SEQ ID NO: 79, Gong cannot anticipate the subject matter of claim 1 (or claims 11, 66-68, 70, 73-76, and 81-82 dependent thereon).

Accordingly, the rejection of claims 1, 11, 66-68, 70, 73-76, and 81-82 as anticipated by Gong is improper and should be withdrawn.

The rejection of claims 1, 11, 66-68, 70, 73-76, and 81-82 under 35 U.S.C. § 102(b) as anticipated by Monson et al., "The FixL Protein of *Rhizobium meliloti* Can Be Separated Into a Heme-Binding Oxygen-Sensing Domain and a Functional C-Terminal Kinase Domain," *Proc Natl Acad Sci USA* 89:4280-4284 (1992) ("Monson"), is respectfully traversed.

Monson identifies the regions of the FixL protein of *Rhizobium meliloti* required for heme-binding and kinase activity (Monson at 4280, rt. col., 2nd full para.), using recombinantly prepared FixL deletion derivatives (*see* Monson at Fig. 1, and Materials and Methods, pp. 4280-4282). It is the position of the U.S. Patent and Trademark Office that the FixL protein of Monson has a heme binding domain at the N-terminus and an aerotaxis signaling domain at the C-terminus. As described above, FixL proteins belong to the gene regulation subfamily, not the aerotaxis family of oxygen sensors. Furthermore, Monson specifically teaches that FixL contains three distinct domains. The first is an N-terminal domain consisting of four transmembrane helices (4283, rt. col., 2nd full para.). The second is

a region of the FixL protein from residue 127 to residue 260 that binds heme and functions in oxygen binding (*id.*). The third is the C-terminal region of FixL, beginning at residue 260, which contains a functional kinase domain (4280, rt. col., 2nd full para.; *see also* abstract and 4282, rt. col., 2nd full para.). Nowhere does Monson teach or suggest the heme binding protein as recited in claim 1 as part of an isolated complex. Because the FixL protein of Monson does not contain an aerotaxis signaling domain, let alone an aerotaxis signaling domain that has at least 30% identity to SEQ ID NO: 79, Monson cannot anticipate the subject matter of claim 1 (or claims 11, 66-68, 70, 73-76, and 81-82 dependent thereon).

Therefore, the rejection of claims 1, 11, 66-68, 70, 73-76, and 81-82 as anticipated by Monson is improper and should be withdrawn.

The rejection of claims 1, 11, 48-49, 51-54, and 66-82 under 35 U.S.C. § 112 (1st para.) for failure to comply with the written description requirement is respectfully traversed in view of the above amendments.

With respect to the rejection of claims 1, 11, and 66-82, applicants submit that the amendments to claim 1 overcome this rejection. As recited in claim 1, the heme binding domain of the heme binding protein "has at least 20% identity to SEQ ID NO: 76, comprises proline at a position corresponding to residue 37 of SEQ ID NO: 76, phenylalanine at a position corresponding to residue 43 of SEQ ID NO: 76, and histidine at a position corresponding to residue 93 of SEQ ID NO: 76, and associates with the porphyrin." Also recited in claim 1, the aerotaxis signaling domain "has at least 30% identity to SEQ ID NO: 79."

The present application discloses a new class of heme-binding proteins: a myoglobin-like heme binding protein which reversibly binds oxygen with a low affinity, binds diatomic oxygen through the prosthetic group, and triggers a negative aerotactic response (pg. 6, lines 3-6). In particular, the present application demonstrates that such proteins can be complexed with a porphyrin.

Furthermore, the specification teaches in detail the relationship between the structural and functional characteristics of the components of the claimed invention. For example, the present application teaches that specific regions within the heme-binding domains of the heme binding protein (that can form the complex) are highly conserved among known heme-binding proteins (pg. 6, line 30 to pg. 7, line 6, and Figures 1A-B). The specification teaches at page 6, line 30 to pg. 7, line 5, that the residues absolutely conserved among all globins are the proximal His in the F helix (F8) and Phe in the CD region (CD1).

Highly conserved residues include the distal His in the E helix (E7), Phe in the CD4 region, and Pro at the beginning of the C helix (C2). Three of these residues (Pro in C2, Phe in CD1, His in F8) are conserved in both HemAT-proteins of the present invention (identified by asterisks in Figure 1A), and correspond to positions 37, 43, and 93, respectively, in SEQ ID NO: 76. Just as these structural features suggested to the applicants that these new proteins are "heme-containing proteins that generate signals in response to binding of oxygen," one skilled in the art, having read the instant application, would have understood that these highly conserved amino acids represent structural characteristics that correlate to the function of heme binding protein of the complex of the present invention.

In addition, the specification teaches that the C-terminus, i.e., the signaling domain of the claimed complex, has high homology with the signaling domain of bacterial methyl-accepting chemoreceptors, which are known to mediate aerotaxis (pg 6, lines 3-15). More specifically, the C-terminus of the heme-binding protein was identified as the product of an open-reading frame encoding a protein with marked similarities to methyl-accepting chemotaxis proteins ("MCP"), having 30% identity to the cytoplasmic signaling domain of Tsr (SEQ ID NO: 79), an MCP from *Escherichia coli* (pg. 7, lines 20-29 and Figure 1B). Furthermore, it was known in the art at the time the present invention was made that a high degree of amino acid homology existed among chemotactic proteins. For example, Tsr contains a highly conserved region in the C-terminal domain (designated "HCD" in Figure 1B of the specification) that is present in the C terminus of all chemotactic receptors (*see* Rebbapragada et al., "The Aer Protein and the Serine Chemotactic Tsr Independently Sense Intracellular Energy Levels and Transduce Oxygen, Redox, and Energy Signals for *Escherichia coli* Behavior," *Proc Natl Acad Sci USA* 94:10541-10546 (1997), at pg. 10542, left col., 4th full para., attached hereto as Exhibit 3). Thus, from the teachings of the present application and the general knowledge in the art, one skilled in the art would have understood what structural characteristics would produce a heme binding protein as recited, and which can be complexed with a porphyrin.

With respect to claims 48, 49, and 51-54, applicants submit that the amendments to claim 48 overcome this rejection. As recited in claim 48, the heme binding domain that forms part of the chimeric protein "has at least 20% identity to SEQ ID NO: 76, and comprises proline at a position corresponding to residue 37 of SEQ ID NO: 76, phenylalanine at a position corresponding to residue 43 of SEQ ID NO: 76, and histidine a position corresponding to residue 93 of SEQ ID NO: 76." For the reasons noted above,

persons of skill in the art were well aware of the conserved features of signaling domains of chemotaxis receptors, and therefore would have appreciated that other signaling domains could be combined with the heme binding domains, as described in the present application, to form the claimed chimeric proteins. Because of the known conservation among such signaling domains, persons of skill in the art would also have predicted the functionality of such chimeric proteins.

For all the foregoing reasons, a skilled scientist, having read the present application, would have understood that the inventors were in full possession of the invention as claimed. Accordingly, the rejection of claims 1, 11, 48-49, 51-54, and 66-82 under 35 U.S.C. § 112 (1st para.) should be withdrawn.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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